UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/15
2[illegible] October 1974

Original : ENGLISH

Division of Narcotic Drugs

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM

No. 15

Report of the Third Working Group on Papaver bracteatum [1]

Beltsville, Maryland, United States of America

7 - 11 October 1974

1/ This meeting was held with the financial support of the United Nations Fund for Drug Abuse Control.

GE. 74-10622

REPORT OF THE THIRD WORKING GROUP ON PAPAVER BRACTEATUM

1. During recent years, considerable interest has been expressed in Papaver bracteatum as a possible source of thebaine and thus, by conversion, of codeine[2]. A collaborative research project on this poppy was initiated by the United Nations Narcotics Laboratory and investigations are being carried out in many countries. Working Groups of experts on Papaver bracteatum were held in Geneva[3] (December 1972) and in Teheran[4] (September 1973).

2. The third Working Group of experts[5] was convened in Beltsville, Maryland, United States of America, from 7 to 11 October 1974, with the financial support of the United Nations Fund for Drug Abuse Control. Facilities for the meeting were provided by the United States Department of Agriculture, through the courtesy of Mr. T.W. Edminster, Administrator of the Agricultural Research Service, United States Department of Agriculture.

3. The meeting was opened by Mr. L.F. Cavanaugh[6] of the United States Department of State who welcomed the Group and stressed the importance of the research being carried out on Papaver bracteatum.

4. Dr. Q. Jones was elected Chairman by acclamation. Dr. O.J. Braenden represented the United Nations and Miss E.S. Lumsden was the Executive Secretary of the Working Group.

2/ Codeine is the most widely used anti-tussive and it is also extensively used as a mild analgesic. It is recognized that there are shortages in the raw materials for the production of codeine, which is mostly derived at present, by the process of methylation, from the morphine obtained from the opium poppy - about 90% of the legally produced morphine being converted into codeine.

3/ See report, document ST/SOA/SER.J/1.

4/ See report, document ST/SOA/SER.J/2.

5/ The list of participants and observers is given in Annex I.

6/ The text of the opening statement is given in Annex II.

5. The Working Group paid tribute to the memory of the late Professor Celâl Tariman of the University of Ankara, who died in an automobile accident in January 1974 and who had participated in the first two Working Groups on Papaver bracteatum.

6. The list of suggested aspects for discussion[7] that had been prepared by the Secretariat, was adopted.

7. It was noted that considerable progress had been made since the initiation of the research, particularly since the second Working Group.

8. It was reported that intensive field study combined with cytological and chemical analysis of Papaver section Oxytona[8] had resulted in the re-evaluation of species in the section[9]. These biosystematic studies showed that P. bracteatum is relatively uniform in important taxonomic characters and is identifiable by its robust habit, usually several conspicuous floral bracts, dark red, blotched petals[10] and, before flowering, by the erect oblong bud with short, appressed, broad-based bristles on the calyx. The chromosome number of all the individuals examined was 2n = 14. Stoma length and pollen diameter are also characteristic for P. bracteatum. The principal alkaloid is thebaine. In some populations alpinigenine is present in small quantities.

9. Certain plants of P. bracteatum were found in northern Iran containing mixtures of thebaine and isothebaine or thebaine and some minor alkaloids. These plants were considered to be natural hybrids of P. bracteatum with P. orientale and P. bracteatum with P. pseudo-orientale respectively.

10. The occurrence of P. bracteatum in Iraq, Turkey and the Union of Soviet Socialist Republics has been reported, but the Group felt that further confirmation was needed on the total natural distribution of this poppy.

7/ See Annex III.

8/ P. Goldblatt, (1974) Ann. Missouri bot. Gard., 61, 264-269.

9/ P. bracteatum, P. orientale and P. pseudo-orientale. A comparison of the characteristics of these three species is given in Annex IV.

10/ The petals turn dark purple on drying.

11. Research was carried out on the possible correlation between growth characteristics and thebaine content. Various parameters were studied including germination conditions, vigour of seedlings, tendency to flower during first year of growth, colour of foliage, number of capsules per plant, size and shape of capsules and colour of pollen. It seemed that there was no correlation between the thebaine content and any characteristic studied.

12. Studies have shown that maximum germination occurred with constant temperature of 10-25°C; 15°C appeared best for general testing.

13. Seedlings grew well in clay, clay-loam and loam soils but poorly in sandy soils or in soils high in organic matter. Seedlings emerged well from soil depths of 0.5 cm or less. Mulching is useful in preventing desiccation damage to which seedlings are prone. With adequate irrigation, the type of seed bed has little effect on seedling establishment.

14. Investigations were carried out to determine the effect of soil pH on germination and seedling establishment. Good results were obtained for germination and seedling growth from pH 5.1 - pH 10 [11]. The pH of the soil is relatively unimportant within this range, but the salts affecting pH are very important. It was noted that the addition of sodium salts seemed to inhibit germination.

15. Direct seeding in the field was successful. Germination continued in field plantings throughout the growing season. Roots of direct-seeded plants grew deeper than 60 cm during the first growing season. Transplanted plants 4 weeks or older were easily established with plug-plantings and were slightly larger than direct-seeded plants at the end of the first growing season.

16. Considerable information has now been developed on vegetative reproduction of P. bracteatum. It was felt that because of the ease with which direct seeding and seedling transplantation in the field can be accomplished and the apparent uniformity of high thebaine yields in plants derived from seed, vegetative propagation would not be necessary in commercial growing of this plant.

11/ The Group realized that soils of pH 10 were rarely, if ever, found in nature.

17. The irrigation requirements of P. bracteatum vary with the type of soil, amount of wind and prevailing temperatures. However it was noted that after the plants were established the water requirements were low compared with other crop plants since P. bracteatum derives moisture from deeper soil layers.

18. Experiments on self-pollination had been successful in some areas and viable seed had been obtained by bagging the opening bud with a paper bag, removing it as soon as the petals fall. However, other investigations of this nature had not been successful.

19. Pest control was a problem - particularly in connexion with weeds. Asulam[12/] can be an effective herbicide for controlling weeds in P. somniferum. Some studies show that it can also be effective in the case of P. bracteatum (except for chenopods). A promising new herbicide, glyphosate, is being tested for weed control in P. bracteatum. Further research is needed to evaluate a wide range of herbicides under many different conditions. The effects of herbicides on the alkaloid profile of P. bracteatum will have to be determined.

20. During intensive experimental cultivation it has been found that, in some localities, P. bracteatum is susceptible to plant diseases and to damage from insects. These problems require further study.

21. Research is needed to determine the most suitable time and methods of harvesting. It was suggested that harvesting at the time of maximum thebaine content of the capsules may not necessarily be the most economical. It may be economically more feasible to harvest after full seed maturity avoiding drying costs and obtaining seed of higher quality. Research will be carried out to determine which portions of the plant should be harvested and the best methods for doing so. The Group felt that detailed consideration of harvesting should be deferred until a future working group.

12/ methyl sulfanilylcarbamate.

22. During 1974, P. bracteatum was cultivated experimentally in many countries, particularly in Australia, the Democratic Republic of Germany, France, Hungary, India, Iran, Ireland, Israel, Japan, The Netherlands, Norway, Poland, Switzerland, United Kingdom, United States of America and Yugoslavia. In this connexion, general appreciation was expressed for the seeds which had been provided by Professor Lalezari of Teheran University through the United Nations Narcotics Laboratory.

23. The thebaine content in the various organs of P. bracteatum plants grown from Iranian and other seeds in various countries had been determined: roots 0.17 - 0.69%; stems 0.3 - 1.0; leaves 0.099 - 0.45%; petals 0.85 - 1.15%; bracts 2.12%; stigmas 0.55 - 0.75%; capsules 0.30 - 5.8%. Although there was considerable variation from plant to plant and between strains, it was noted that Arya II [13/] material gave consistently higher and more uniform values for the thebaine content in the various plant parts.

24. Variations had been reported in the thebaine content in the plant according to the time of day. It was suggested that these might be due to changes in moisture content and not to changes in the absolute amount of thebaine. In P. bracteatum straw, the thebaine content remains constant for long periods of storage.

13/ At the second Working Group on P. bracteatum considerable interest was expressed in new collections from the Mahabad region in the west of Iran. The capsules of these plants have the highest content of thebaine. The pigment and resin content is low and no other alkaloid was detected in the dry capsules. It was thus possible to obtain pure thebaine relatively easily. In view of the importance of this discovery, the second Working Group felt that it would be desirable to nomenclaturally distinguish collections of the Alborz Mountains from those of the Mahabad region by referring to the former as "Arya I" and to the latter as "Arya II". (See document ST/SOA/SER.J/2.)

25. In considering methods for the determination of thebaine in P. bracteatum, the Group felt that several reliable methods were available. However, to facilitate inter-laboratory comparison, it was desirable that a uniform procedure should, in addition, be used in all laboratories. A Sub-Group, under the chairmanship of Professor J.M. Fairbairn considered this question. The conclusions and recommendations of the Sub-Group are given in Annex V.

26. Reference was made to the use of high pressure liquid chromatography techniques for the determination of thebaine. This had the advantage that a simple extract of the sample could be injected directly into the chromatograph. The method was very rapid and the sample required little handling.

27. Where large numbers of analyses were needed, it was desirable to have a rapid method with sufficient accuracy to classify samples according to their relative rather than their absolute thebaine value.

28. In determining the thebaine content of capsules it would be of value to record the developmental stage (days from flower opening) of the capsules analysed.

29. Further investigations were also necessary on the stability of thebaine during the extraction process and storage.

30. The Group felt that the reference sample of capsule material which had been prepared by the United Nations Narcotics Laboratory had been of great value in comparing methods for the determination of thebaine. It was suggested that reference samples should also be prepared of leaf, stem and root tissue.

31. Further research was needed on the biosynthesis, translocation and metabolism of thebaine. The action of enzymes during biosynthetic and degradation processes should also be studied.

32. It had been reported earlier that thebaine occurs in the seeds of P. bracteatum. This was probably due to the contamination of the seeds by the latex as it had not been possible to confirm the presence of thebaine.

33. On the basis of present knowledge, there seems to be little qualitative difference between the oil obtained from P. bracteatum seeds and that from P. somniferum. Data on the oil content and fatty acid composition of P. bracteatum seeds are given in Annex VI.

34. Remarkable similarities were found in the amino acid content of the seed proteins from these poppies. Of the 30 identified amino acids examined, only one significant difference was noted. Twice the amount of methionine 14/ was found in the case of P. bracteatum than in P. somniferum.

35. The Group felt that further research, including animal feeding tests, should be carried out to determine whether P. bracteatum seed oil and meal were suitable for human and/or animal consumption.

36. In summarizing its discussions, the Working Group recommended that during the coming year special attention should be given to research in the following areas:

- weed control: cultural, chemical;
- detailed studies, in geographic areas of potential commercial production, of light, thermal and other ecological factors for maximizing thebaine production from P. bracteatum. These studies will pay special attention to such characteristics as first season flowering, non-shattering capsules and water-resistant capsules;
- in addition to the reference sample of capsule material which has been provided to participants, similar reference samples should be prepared of root, stem and leaf tissue for comparing results obtained by various analytical methods;
- rough screening method for thebaine determination;
- biosynthesis, translocation and metabolism of thebaine;
- disease control: natural resistance, cultural methods, pesticides;

14/ This is of special significance because plant proteins are usually deficient in this essential amino acid.

- what to harvest, when and how for best economic returns;
- selection and breeding for plant improvement;
- seed oil and protein evaluation for food and feed;
- influence of precursors of plant growth regulators (hormonal type) and trace elements upon alkaloidal patterns and content of the plant;
- selective field collecting of additional P. bracteatum germ plasm.

37. Recognizing the importance of the role of the United Nations Laboratory in the research on P. bracteatum, the Group recommended that it continue its activities in the coordination of research and the exchange of information. It was also recommended that close contact should be maintained between the U.N. Laboratory and scientists collaborating in the research.

38. The Group considered that it would be desirable to have a further meeting in 1975. General appreciation was expressed for the invitation of Mr. J. Morice, Director of the Station d'Amélioration des Plantes, Rennes, to hold the fourth Working Group on Papaver bracteatum in France.

ANNEX I

THIRD WORKING GROUP ON PAPAVER BRACTEATUM

Participants

Dr. C.B. COFFMAN,
Agronomist,
Agricultural Research Center (West),
U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.

Dr. A.M. DAVIS,
Research Agronomist,
Agricultural Research Service,
U.S. Department of Agriculture,
Pullman, Washington, U.S.A.

Dr. J.A. DUKE,
Chief, Plant Taxonomy Laboratory,
Plant Genetics and Germplasm Institute,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.

Dr. H.H. EL-HAKIM,
General Manager,
Pharmaceutical Administration and Control Laboratories,
Ministry of Health,
Cairo, Arab Republic of Egypt.

Professor J.W. FAIRBAIRN,
Head, Department of Pharmacognosy,
School of Pharmacy,
University of London,
29/39 Brunswick Square,
London WC1N 1AX, United Kingdom.

Dr. W.A. GENTNER,
Plant Physiologist,
Agricultural Research Center (West),
U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.

Dr. N.W. GILBERT,
Research Agronomist,
Agricultural Research Service (Western Region),
U.S. Department of Agriculture,
Rocky Mountain Forest & Range Experimental Station,
Flagstaff, Arizona 86001, U.S.A.

Dr. T.N. JOHNSEN, Jr.,
Research Leader,
Agricultural Research Service,
U.S. Department of Agriculture,
Rocky Mountain Forest & Range Experimental Station,
Flagstaff, Arizona 86001, U.S.A.

Dr. Q. JONES,
Staff Scientist,
Plant Introduction and Narcotics,
Agricultural Research Center (West),
U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.

Dr. J. KRZYMANSKI,
Department Chief,
Institute of Plant Breeding and Acclimatization,
ul Sieroca 1 a,
61-771 Poznan, Poland.

Professor I. LALEZARI,
Dean of the Faculty of Pharmacy,
University of Teheran,
P.O. Box 12-1317,
Teheran, Iran.

Professor D. LAVIE,
Department of Organic Chemistry,
The Weizmann Institute of Science,
Rehovot, Israel.

Mr. J. MORICE,
Directeur,
Station d'Amélioration des Plantes,
65, rue de Saint-Brieuc,
35042-Rennes, France.

Dr. K. NISHI,
Technical Official,
Kasukabe Experimental Station of Medicinal Plants,
National Institute of Hygienic Sciences,
30 Kasukabe, Kasukabe-Shi,
Saitama-Ken, Japan.

Professor A. NORDAL,
Head, Department of Pharmacognosy,
Institute of Pharmacy,
University of Oslo,
Postboks 1068, Blindern,
Oslo-3, Norway.

Dr. V.S. RAMANATHAN,
Chief Chemist,
Central Revenues Control Laboratory,
Hill Side Road,
New Delhi-110012, India.

Professor C.A. SALEMINK,
Head, Department of Organic Chemistry of Natural Products,
Director of the Organic Chemical Laboratory,
University of Utrecht,
Croesestraat 79,
Utrecht, The Netherlands.

Professor K. SCHREIBER,
Director,
Institute of Plant Biochemistry of the Academy of Sciences
of the German Democratic Republic,
Weinberg,
402-Halle/Saale, Democratic Republic of Germany.

Dr. S.P. SOBOL,
Laboratory Director,
Special Testing and Research Laboratory,
Drug Enforcement Administration,
McLean, Virginia 22101, U.S.A.

Dr. P. TETENYI,
Directeur,
Institut de Recherche sur les Plantes médicinales,
H-2011 Budakalász, Hungary.

Professor O. TOSUN,
Chairman, Plant Growing and Breeding Department,
Ziraat Fakültesi,
Ankara University,
Ankara, Turkey.

Dr. P.G. VINCENT,
Research Plant Physiologist,
New Herbicide Explorations and Chemonarcocide Laboratory,
Pesticide Action Laboratory,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.

Observers

Mr. C.F. BAKER,
Vice President - Corporate Planning,
Burroughs Wellcome Company,
Research Triangle Park, North Carolina 27707, U.S.A.

Dr. H.H. BOSMAN,
Deputy General Manager,
Verenigde Pharmaceutische Fabrieken N.V.,
Kloosterstraat 6,
Oss, The Netherlands.

Dr. B.B. BROWN,
Director of Research,
S.B. Penick & Company,
100 Church Street,
New York, N.Y. 10007, U.S.A.

Mr. C. CANDILLON,
Directeur,
Service Agricole,
Francopavot,
94, rue de Paris,
91300-Massy, France.

Mr. V.V. DOBROHOTOFF,
Director,
Marketing & Sales,
Merck & Co. Inc.,
Rahway, New Jersey 07065, U.S.A.

Dr. N.J. DOORENBOS,
Chairman, Department of Pharmacognosy,
School of Pharmacy,
University of Mississippi,
University, Mississippi 38677, U.S.A.

Mr. J. FROMUTH,
Director of Development, Papaver bracteatum,
Mallinckrodt Chemical Works,
Mallinckrodt & 2nd Streets,
St. Louis, Missouri, U.S.A.

Dr. E.L. GREW,
Head, Research and Development,
Macfarlan Smith Ltd.,
Wheatfield Road,
Edinburgh EH11 2QA, United Kingdom.

Dr. L.O. HOPKINS,
Head, Organic Chemistry Research,
Institute for Industrial Research and Standards,
Ballymun Road,
Dublin-9, Ireland.

Dr. M.H. JOFFE,
Special Assistant,
National Institute on Drug Abuse,
11400 Rockville Pike,
Rockville, Maryland 20852, U.S.A.

Mr. C.A. LOPEZ,
Consultant,
S.B. Penick & Co.,
408 Hunt Lane,
Manhasset, N.Y. 11030, U.S.A.

Mr. M. NEDELCEV,
Director of Alkaloids Production,
"Alkaloid" Chemical Pharmaceutical Cosmetic Industry,
P.O. Box 8,
Skopje, Yugoslavia.

Mr. S. SAMUELSON,
Food and Agriculture Officer,
U.S. A.I.D.,
Ankara, Turkey.

Dr. E.F. SCHOENEWALDT,
Manager,
Process Research,
Merck & Co. Inc.,
Rahway, New Jersey 07065, U.S.A.

Dr. W.F. SMITH,
Research Biologist,
Agricultural Chemicals Division,
Biochemical Department,
E.I. du Pont de Nemours & Co. Inc.,
Wilmington, Delaware 19898, U.S.A.

Dr. J.A. SNYDER,
Market Research Manager,
Pharmaceuticals Division,
Biochemicals Department,
E.I. du Pont de Nemours & Co. Inc.,
Wilmington, Delaware 19898, U.S.A.

Mr. R. STRATMEYER,
General Manager - Narcotics,
Mallinckrodt Chemical Works,
P.O. Box 5439,
St. Louis, Missouri 63147, U.S.A.

Mr. W.S. VALLANCE,
Manager,
Abbott Universal (Pacific and Far East Operations),
140 Arthur Street,
North Sydney, N.S.W. 2060, Australia.

Mr. Olaf WEIDER,
Managing Director,
Weiders Farmasøytiske A.S.,
Hausmannsgt. 6,
Oslo-1, Norway.

ANNEX II

THIRD WORKING GROUP ON PAPAVER BRACTEATUM

Opening statement made by the
Representative of the United States Department of State,
Mr. L.W. Cavanaugh

It is an honour for me, on behalf of the Department of State, to welcome you all to this meeting on *Papaver bracteatum*.

We believe this gathering is an especially significant one, because a good deal of the credit for the current interest in, and promise of *Papaver bracteatum*, as an alternate source of raw material for codeine, belongs to the Group assembled here.

Under the sponsorship of the United Nations Narcotics Laboratory, at least fifteen countries are cooperating in the research needed to evaluate this plant and its useful products. Without this international cooperation we would likely have had fifteen isolated research efforts with information exchanges having to take the time-consuming route of awaiting publication of research results in many different scientific journals, in many different languages. Through the meetings of this Working Group and the rapid publication avenue provided by the United Nations Narcotics Laboratory, the time frame for this information exchange has been drastically shortened. I believe it is obvious that this has helped all concerned in avoiding unproductive lines of research and in focusing resources on critical problem areas.

The result has been a cooperative effort accomplishing, in less than three years, what would have taken several times as long through uncoordinated efforts by the same number of scientists.

We congratulate the United Nations Narcotics Laboratory for its role as a catalyst in this programme and we wish to express our appreciation to each of you for your contributions to advancing the world community's knowledge of *Papaver bracteatum*.

Please accept our very best wishes for a successful meeting.

ANNEX III

THIRD WORKING GROUP ON PAPAVER BRACTEATUM

Suggested aspects for discussion

Review of present knowledge of Papaver bracteatum (with special reference to research carried out during 1974) and preparation of guidelines for future research. Consideration of the following broad categories:

- agronomy of P. bracteatum;
- chemistry of P. bracteatum;
- utilization of P. bracteatum.

ANNEX IV

Comparison of characteristics of Papaver bracteatum, Papaver pseudo-orientale and Papaver orientale */

Papaver bracteatum	Papaver pseudo-orientale	Papaver orientale
Robust plant of relatively dry slopes, 1,500 to 2,500 m elevation	Robust plants along streambanks, seepage or alluvial areas 1,500 to 2,000 m	Slender plants; of moist or alpine slopes, 1,800 to 3,000 m
Stem 60-120 cm tall, appressed hispid above (spreading in lasiothrix); leaves to 45 cm long, pinnate to pinnatisect, dentate to bidentate	Stem to 80 cm long, somewhat intermediate in pubescence, leaves to 45 cm long, pinnatisect, dentate	Stem 40-100 cm tall, spreading-hispid-villose below; appressed above; leaves to 35 cm long, lacerate to shallowly pinnatisect, dentate
Cauline leaves 5-7, uppermost on upper third of stem	Cauline leaves 5-6, uppermost on upper third of stem	Cauline leaves 2-3, rarely above the middle of stem
Stomatal pores 20-32 microns long	Stomatal pores 43-60 microns long	Stomatal pores 32-45 microns long
Flowers 1-20, the buds not drooping, oblong to obovate, the sides nearly parallel, the apex truncate; peduncle 3-4 mm thick	Flowers 1-10; the buds erect or drooping, obovate, as in orientale	Flowers 1-4, buds drooping, rounded at top, the sides not parallel, peduncle 2-3 mm thick
Bracts (0)-5-8, mostly calyx like, 1-5 cm long, entire to serrate or lobate	Bracts 0-4, entire to dentate	Bracts usually absent; peduncles free of leaves for some distance

*/ This summary was compiled by James A. Duke, Plant Taxonomy Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Service, United States Department of Agriculture.

Papaver bracteatum	Papaver pseudo-orientale	Papaver orientale
Sepal hairs appressed, conical, somewhat 3-cornered at the base	Sepal hairs slender and spreading as in orientale	Sepal hairs spreading, cylindrical, round at the base
Petals usually 6, to 10 cm long; sepals 3	Petals 4 or 6, sepals 2 to 3	Petals usually 4, to 9 cm long, sepals 2
Petals scarlet, with longitudinal black blotches near base, drying deep wine purple	Petals orange-scarlet, with rectangular black blotches (sometimes unmarked), drying purplish to pinkish brown	Petals pale orange without maculations, drying pale pinkish brown
Filaments to 1.5 cm long; anthers linear, purple, 2.8-5.5 mm long; pollen 24-27 microns in diameter	Filaments to 1.3 cm long; anthers linear, purple, 1.7-3.5 mm long; pollen 27-31 microns in diameter	Filaments to 1 cm long; anthers oblong, yellow to violet, 1.8-3 mm long; pollen 26 to 29 microns in diameter
Capsules 0.5-3 cm long, 1-4 cm broad, the tops convex, flat, or concave, not changing with age; rays 10-24, the teeth acute	Capsules 0.5-2.5 cm long, the tops flat; rays 9-19	Capsules 0.5-2.0 cm long, starting convex, becoming flat with age; rays 8-15, the teeth obtuse
Rosette seedling leaves more dissected, serrate, hairy, less glaucous	Rosette seedling leaves very much like bracteatum, hairy but not glaucous	Rosette seedling leaves less dissected, serrate and hairy, more glaucous
Haploid chromosome number 7	Haploid chromosome number 21	Haploid chromosome number 14
Thebaine present, regardless of minor alkaloidal variance; alpinigenine frequent	Isothebaine, traces of thebaine and other alkaloids present	Thebaine usually if not always absent, oripavine often dominant, with isothebaine secondary

ANNEX V

Report of the Sub-Group on Methods for the Determination of Thebaine

(Chairman - Professor J.W. Fairbairn; Secretary - Dr. P.G. Vincent)

The objectives of the Sub-Group were to provide general guidelines for the analyses of tissue from P. bracteatum that would be compatible with the laboratory facilities of all*/participating members. Criteria for the guidelines included that the method be simple, accurate and cover a wide range of plant material and quantities of thebaine.

1. Drying of Plant Tissue:

It was recommended that plant tissue be oven dried at 60°C to a constant weight as soon as possible following collection. Several members took exception to this procedure noting that thebaine may be lost using this protocol and that freeze-drying of samples would possibly be better. However, not all members had access to freeze-drying equipment. It was suggested that tissue be cut-up to facilitate oven drying, especially root tissue.

2. Particle Size:

It was recommended that samples be powdered so that all the sample passes through a 0.5 mm mesh and is thoroughly mixed immediately before weighing for analysis. Weight correction due to hygroscopic water absorption should be made by heating a small portion to 105°C and re-weighing at the time of analysis.

3. Sample Size for Analysis:

It was recommended that 0.1 to 0.5 grams of sample be used in analyses.

4. Extraction Solvent:

Acetic acid (5%) was suggested as the extraction solvent but would be considered tentative until fully evaluated by the members.

---

*/ Not all members were equipped to participate in a total refereed quantitative study.

5. Procedure:

(a) Samples should be agitated for 30 minutes in 25 ml of acetic acid (5%) solution. The Sub-Group recommends that shorter or longer agitating times be evaluated for efficacy of extraction technique.

(b) Filter eluent through a sintered glass funnel and repeat extraction two further times. Combine the filtrates.

(c) Add 25 ml of $CHCl_3$ and adjust the pH of the aqueous phase to pH 9.5-10 with concentrated NH_4OH with agitation. Separate $CHCl_3$ phases.

(d) Repeat extraction of the aqueous phase with one 25 ml portion of $CHCl_3$ followed by two 15 ml portions of the same solvent.

(e) Combine the $CHCl_3$ phases and evaporate to dryness preferably in vacuo at 40°C.

(f) To the flask containing the residue, add a known volume of ethanol containing an appropriate quantity of internal standard. Suggested internal standards include tetrahydrothebaine, cholesterol acetate, antipyrine and laudanosine. Use of one or more internal standards may be appropriate for certain analytical applications.

6. Gas-liquid Chromatography:

The Sub-Group recommended that deactivated glass columns or glass-lined steel columns be used to avoid decomposition of thebaine (via metal catalysis). It was suggested that the columns be packed with 100/120 mesh Gas Chrom Q coated with 2-3% OV-17 liquid phase or their equivalents. Suggested operating temperatures should range between 260-280°C with either nitrogen or helium as carrier gas.

7. Results:

Results should be calculated on the basis of moisture-free content of plant tissue after drying at 105°C. */

Results from the above procedure should be evaluated using plant material designated UNB-17 and other reference samples and data forwarded to the United Nations Narcotics Laboratory by the end of February 1975.

---

*/ Thebaine standardization: check the purity of the standard thebaine by determining the $E^{1\%}_{1\,cm}$ in 0.1N HCl at 285 nm or at the λ max.

ANNEX VI

Analysis of seed samples of P. bracteatum

The oil content was estimated by NMR and the fatty acid composition by gas chromatography.

	UNB-4	UNB-18	UNB-21
oil content	40.2%	36.2%	43.3%
fatty acid composition			
palmitic acid	8.0%	7.1%	7.9%
palmito-oleic acid	0.3%	0.3%	0.5%
stearic acid	2.5%	2.5%	2.5%
oleic acid	12.5%	8.7%	12.0%
linolic acid	76.3%	80.8%	76.8%
arachidic acid	0.5%	0.6%	0.7%

Data provided by Dr. J. Krzymanski, Institute of Plant Breeding and Acclimatization, Poznań, Poland.